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(54) Title: IMMUNOSUPRESSION WITH ANTI-PAN T-CELL IMMUNOTOXIN COMPOSITIONS

(57) Abstract

Improved immunosuppression for use in antigenic biological response modifier therapies is provided by the concurrent administration of T-cell specific immunotoxins as an immunosuppressive agent. This immunosuppressive immunotoxin can comprise an anti-Pan T-cell immunoglobulin complexed with a cytotoxic agent, such as a ribosomal inhibiting protein. Compositions comprising the immunosuppressive immunotoxin with other immunosuppressive agents have been developed to permit multiple-dose regimens or extended therapies with the modifiers.

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IMMUNOSUPPRESSION WITH ANTI-PAN T-CELL IMMUNOTOXIN COMPOSITIONS

Field of the Invention

This invention relates generally to methods for improving the effectiveness of immunogenic substances, such as proteins, in various human treatment applications and, more particularly, to the concurrent administration of T-cell specific immunotoxins as an immunosuppressive agent to permit extended treatment regimes with monoclonal antibodies, immunotoxins, recombinantly-produced proteins and the like in cancer and other therapies.

Background of the Invention

The development of hybridoma technology by Kohler and Milstein in 1975 was heralded as a major technology breakthrough for the fields of immunology and medicine. For the first time, researchers were able to transform B-lymphocytes to create hybrid cells, with immortal potential, capable of secreting monoclonal antibodies, i.e., a single species of antibody reactive with a single type of epitope on a selected antigen. In practice, however, applying monoclonal antibody technology to improve chemotherapeutic and other therapies has been difficult. In the decade since the discovery, few therapeutic successes have been reported, despite extensive research efforts.

One reason for the lack of successful human therapeutic treatment regimes, at least in tumor therapy, is the fact that the mere binding of a monoclonal antibody to a target cell frequently does not induce cell death. To overcome this problem, there were considerable efforts devoted to coupling monoclonal antibodies to various cytotoxic agents

capable of killing targeted cells. The monoclonal antibody would act as a "magic bullet", delivering the cytotoxic agent specifically to the desired cell. These immunoconjugates are known as "immunotoxins."

While treatment studies with immunotoxins have been widespread and to some extent successful, the use of immunotoxins is often curtailed by a patient's immune response to both the monoclonal antibody and the toxin components of the immunotoxin. It is particularly thought to be a problem when the monoclonal antibody is of mouse or other non-human origin, although a patient's production of anti-idiotype or anti-allotype antibodies would cause significant diminishment of the usefulness of immunotoxins even when made with human monoclonal antibodies.

An immune response against an immunotoxin can cause premature removal of the immunotoxin from the patient's serum, significantly limiting the immunotoxin's effectiveness. Multiple treatment regimes are extremely susceptible to this problem, and significantly increasing the immunotoxin dosage is generally undesirable, in part because the patient may suffer allergies and other harmful effects of immune reactions against the immunotoxin.

The advent of recombinant DNA technology has provided a vast arsenal of novel therapeutic proteinaceous agents (including new forms of immunotoxins) for treating a whole array of disease states. Unfortunately, many of these agents are highly antigenic, severely limiting their effectiveness. Attempts to minimize the proteins' immunogenicity, such as with administration of immunosuppressive agents, often results in serious side effects (e.g., nonspecifically killing or inhibiting white blood cells). This presents a particular problem in debilitated patients.

In general, it is now known that the immune system comprises cellular and humoral components, both

of which operate under positive and negative feedback systems. Any modification of an immune response against a therapeutic agent must be carefully controlled so as to not destroy the patient's immune competence as a whole.

Thus, there is significant need for new methods of specifically abrogating the host immune response against antigenic therapeutic agents, or diminishing it to a level such that extended treatments are feasible. These methods of treatment should not include regimes that are incompatible with the therapeutic agents or that will increase the amount of agent required to be administered to the patient. Ideally, the new treatment methods will not substantially increase the toxicity of the overall therapy. The present invention fulfills these needs.

SUMMARY OF THE INVENTION

The present invention provides novel methods for enhancing the effectiveness of biological response modifiers in therapy on human patients, which methods comprise concurrently administrating with the modifiers an immunosuppressive dose of an immunotoxin comprising an anti-pan T-cell monoclonal antibody, such as those reactive with the CD3, CD5 or CD7 antigen clusters. This novel treatment has particular applicability in multiple dose treatment regimens using proteinaceous biological response modifiers, such as in chemotherapy when utilizing multiple injections of a primary immunotoxin comprising a cytotoxic agent conjugated to monoclonal antibodies specifically reactive with melanoma or other tumor associated antigens. The immunosuppressive immunotoxin can be administered concomitantly with the response modifier, e.g., with primary immunotoxins, to achieve a reduced immune response against both the immunoglobulin and toxin components of the immunotoxin.

The delivery component of the immunosuppressive immunotoxin may comprise one pan T-cell reactive immunoglobulin or a collection of immunoglobulins reactive with a plurality of T-cell markers, such as those associated with antigen clusters CD2, CD3, CD4, CD5, CD6, CD7, CD9, CD11 and CD45R. The cytotoxic agent component of the immunotoxin is preferably a ribosomal inhibiting protein, such as ricin or ricin A-chain. Various administration intervals and dosages that produce a substantially decreased immune response to the desired antigen may be utilized.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Novel methods are provided for improving immunogenic biological response modifier therapy in human patients, by inhibiting the patient's immune response against the modifier through the co-administration of at least one immunosuppressive dose of an immunosuppressive immunotoxin capable of neutralizing mature T-cell activity. The immunotoxin treatment diminishes the patient's immune response against antigenic components of the modifier. Thus, for example, the patient's immune response would be reduced against both the delivery component and the toxic component of a primary immunotoxin, thus prolonging the efficacy of the primary immunotoxin and enhancing the prognosis for recovery. In multiple treatment regimens, use of an immunosuppressive immunotoxin avoids raising subsequent doses of the primary immunotoxin or other response modifier.

As used herein, the term "biological response modifier" refers to those immunogenic agents capable of altering the natural host response to a disease state. This term is intended to encompass pharmaceutical substances, typically antigenic, produced by traditional purification and chemical modification procedures, as well as those produced through the use

of recent developments in molecular biology, recombinant genetics, hybridoma technology and the like. In this context, representative modifiers include endotoxin-type inhibitors, immune RNAs and other non-protein antigenic immunomodulating agents, but more typically include any of the various well-known proteinaceous therapeutic substances such as lymphokines, colony stimulating factors, interleukins, growth factors and hormones (EGF, FGF, etc.), thymosins, and, of course, immunoglobulins.

Also, as used herein, the term "primary immunotoxin" refers to the immunotoxin responsible for treating a specific disease. In comparison, an immunosuppressive immunotoxin is used to limit a patient's response against the primary immunotoxin in accordance with the teachings of the present invention.

Immunotoxins are characterized by two components. One component is a cytotoxic agent which is usually fatal to a cell when attached or absorbed. The second component, known as the "delivery vehicle", provides a means for delivering the toxic agent to a particular cell type, such as cells comprising a carcinoma. The two components are commonly chemically bonded together by any of a variety of well-known chemical procedures. For example, when the cytotoxic agent is a protein and the second component is an intact immunoglobulin, such as a monoclonal antibody, the linkage may be by way of heterobifunctional cross-linkers, e.g., SPDP, carbodiimide, glutaraldehyde, or the like. Production of various immunotoxins is well-known within the art, and can be found, for example in "Monoclonal Antibody-Toxin Conjugates: Aiming the Magic Bullet," Thorpe et al, Monoclonal Antibodies in Clinical Medicine, Academic Press, pp. 168-190 (1982), and commonly assigned U. S. Patent Application Serial No. 151,744, filed concurrently

herewith, both of which are incorporated herein by reference.

A variety of cytotoxic agents are suitable for use in immunotoxins. Cytotoxic agents can include radionuclides, such as Iodine-131, Yttrium-90, Rhenium-188, and Bismuth-212; a number of chemotherapeutic drugs, such as vindesine, methotrexate, adriamycin, and cisplatinum; and cytotoxic proteins such as ribosomal inhibiting proteins, pokeweed antiviral protein, abrin and ricin (or their A-chains, diphtheria toxin A-chains, Pseudomonas exotoxin A, etc.). (See generally, "Chimeric Toxins", Olsnes and Phil, Pharmac. Ther., 25:355-381 (1982), and "Monoclonal Antibodies for Cancer Detection and Therapy," eds. Baldwin and Byers, pp. 159-179, 224-266, Academic Press (1985), both of which are incorporated herein by reference.)

The delivery component of the immunotoxin is typically proteinaceous and can be obtained from a number of sources. Intact immunoglobulins or their fragments, such as Fv, Fab, $F(ab_2)$, etc., or other proteins (i.e., specific binding proteins, such as interleukin-2 (IL-2)) specifically reactive with a selected cell marker (e.g., hormone receptors, such as the IL-2 receptor) are preferably used. Typically, immunoglobulins will be monoclonal antibodies of the IgM or IgG isotype, of mouse, human or other mammalian origin.

A preferred source of monoclonal antibodies is immortalized murine or human cell lines that may be cloned and screened in accordance with conventional techniques. However, recent technical advances have provided additional forms of immunoglobulins and methods of making them. For example, the utilization of recombinant DNA technology has produced functional, assembled immunoglobulins or hybrid chimeric immunoglobulins (e.g., the constant region from human monoclonal antibodies combined with mouse variable

regions), suitable for use in immunotoxins. (See, e.g., EPA 84302368.0, which is incorporated herein by reference.)

In the immunosuppressive immunotoxins of the present invention, the delivery component will be capable of binding to epitopes of markers on selected mature T-cell types, such as inducer T-cells. The marker is generally a unique surface protein, but a variety of markers, such as other proteins, glycoproteins, lipoproteins, polysaccharides and the like, which are produced by the T-cells to be treated, can be utilized in accordance with the present invention. The general immunization fusion, screening, and expansion methods of producing monoclonal antibodies against T-cell markers are well-known to those skilled in the art and can be found, for example, in Goding, Monoclonal Antibodies; Principles and Practice, Academic Press, 2nd Edition (1986), which is incorporated herein by reference.

The T-cell markers most studied at the present time have been grouped into so-called "Clusters of Differentiation," as named by the First International Leukocyte Differentiation Workshop, Leukocyte Typing, Eds. Bernard, et al., Springer-Verlag, N.Y. (1984). This has recently been updated at the Second International Workshop, Leukocyte Typing II (Eds. Reinherz, et al., Springer-Verlag, N.Y. (1986), which is incorporated herein by reference), and Table I presents a list of the better characterized monoclonal antibodies (many of which are available publicly or commercially) reactive with various epitopes on these markers. Additional antibodies discovered to be reactive with these and other T-cell antigens may be utilized for immunotoxins in accordance with the present invention.

TABLE I

Ag Cluster Designation	Antibodies	Leukocyte Subpopulation
CD2	Leu-5b, OKT11, 9.6, T11, 35.1	corticalthymocytes
CD3	Leu-4, OKT3, 38.1, UCH T1, 89b1	Mature T-cells
CD4	Leu-3a, OKT4, 91D6	T helper/inducer cells
CD5	H65, Leu-1, OkT1, T101, T1, 10.2, A50, SCI, HH9	pan T and sub-population of B cells
CD6	OkT12, T411, B614, WT31, MBG6	mature T and sub-population of B cells
CD7	4A, Leu-9, 3A1, C11.3	pan T-cells
CD8	Leu-2, OKT8, OKT5, T8, 51.1, 2D2, C10, UCHT4	cytotoxic and suppressor T-cells
CD45R	Leu-18, 2H4, HB10, HB11, 3AC5	T-cell sub-population

An immunosuppressive immunotoxin composition will typically comprise immunoglobulins (complexed with toxins) that are capable of binding to and removing one or more T-cell subpopulations, preferably mature T-cells. Ideally, the immunoglobulins will only minimally, if at all, cross-react with other leukocyte subsets, particularly pluripotent stem cells.

Thus, in one embodiment of the present invention, an immunosuppressive immunotoxin composition will comprise at least one pan T-cell immunoglobulin reactive agent, e.g., reactive with the CD3, CD5 or CD7 antigen clusters. Alternatively, the immunotoxin composition will be composed of two or more immunoglobulins, each reactive with a different marker of the same or different cell populations to ensure a broad spectrum of T-cell neutralization. Typical combinations will include immunoglobulins recognizing CD4 and CD8; TAC and CD4; or CD7, CD11 and CD5.

A preferred method of measuring the immune response to an immunotoxin is based on the common ELISA assay. Briefly, microtiter plates are coated with the immunotoxin components, i.e., the immunoglobulin and the cytotoxic agent. After standard blocking and washing procedures, appropriate dilutions of patient's serum are added to the plate and any antibodies in the serum binding to the antigens are detected using an alkaline phosphatase conjugated goat anti-human antibody with specificity for IgM or IgG heavy chains. The antibody response is typically reported as a ratio. For each patient, the maximum measurable binding activity following therapy is determined by extrapolating the titration curve to the X axis. The response ratio is defined as a ratio of the titration end point value of the maximum response to the end point value of the patient's pre-treatment (baseline) serum. Other protocols may be substituted to assess the patient's immune response according to means well known by those skilled in the art.

By utilizing each patient's pre-treatment serum as a baseline reference, positive responses to both the immunoglobulin and the cytotoxic agent components of the immunotoxin can be identified. Typically, the humoral aspect of the immune response is analyzed and a wide range of responses to each and/or both of the immunotoxin components is seen.

Based on control response studies and on data from various additional studies, an abrogation or prevention of the development of an immune response is indicated by a ratio less than about 2.0. An acceptable inhibition of a human immune response to either or both components of an immunotoxin would be preferably less than about 5.0 to 10 for both components, most preferably less than about 2.0 to 3.0.

To achieve a suitable reduction of T-cells and, thus, a human immune response, a variety of dosage protocols may be followed, again, depending, e.g., upon the particular primary immunotoxin utilized and the condition of the patient. Obviously, the protocol for administration of the immunosuppressive immunotoxin must be tailored to the primary protocol. The amount of immunosuppressive immunotoxin administered per injection may range from about 0.01 up to about 4.0 mg/kg or more. Larger amounts per injection may be tolerated if the administration schedule calls for a single or a few injections. Lower amounts per injection may be administered over longer time periods of up to two weeks or more. Patient's disease status could influence dose tolerated.

In one dose protocol, between 0.01 and 4.0 mg/kg/day, more preferably from about 0.05 to 1.0 mg/kg/day, and most preferably about 0.1 to 0.5 mg/kg/day of XMMLY-H65-RTA is administered in 14 injections over a period of about 14 days. The initial injections are preferably administered preceding or given concurrently with treatment with the primary

immunotoxin, and the remaining injections administered at regular intervals thereafter.

An immunosuppressive dose would be an amount of immunosuppressive immunotoxin sufficient to limit the patient's immune response to a significantly reduced level of interference with the functional activity of the biological response modifier (e.g., to a level where a subsequent modifier, such as a primary immunotoxin, dose regimen retains substantial efficacy). This corresponds to the inhibiting the T-cell immune response in comparison to a normal response, ideally by about 85% to 95% or more, but inhibition levels of about 50% to 60% may be acceptable in some patients, and at least about 75% inhibition is preferred. Diminishment of various components of the T-cell immune response may be obtained, but reduction in antibody formation is a preferred result.

The period of co-administration of biological response modifiers and immunosuppressive immunotoxin may coincide. In humans, the IgG or IgM response to either the RTA or immunoglobulin portion of an immunotoxin commonly can be detected about 7 to 8 days after exposure to the primary immunotoxin, and peaks at about 15 to 20 days or more thereafter. To minimize this immune response, for example, the immunosuppressive immunotoxin is preferably administered between 3 days before and 3 days after each exposure to the primary immunotoxin. Subsequent doses of the immunosuppressive immunotoxin may be administered to further abrogate this or other aspects of the immune response.

In this manner, prolonged or multiple treatment therapy regimes with the primary immunotoxin (along with the secondary immunotoxin as necessary for immunosuppressors) can be utilized to provide an increased level of therapeutic efficacy. Also, the total primary immunotoxin dose is minimized.

Immunotoxin doses will vary widely according to the treatment.

Various combinations of biological response modifiers and immunosuppressive immunotoxin administration schemes are acceptable for use with susceptible diseases, such as malignant tumors. The disease states most suitable for treatment in accordance with the present invention include neoplasms, particularly those showing retarded growth or total remission when subjected to primary immunotoxin treatment. For example, such treatable neoplasms can include melanomas, gastrointestinal carcinomas, various leukemias, and T-cell and B-cell lymphomas. Aggressive grades of cancers may be particularly suited for treatment with primary immunotoxins. Also, immunotoxins are particularly suited for use as surgical adjuvant.

Those skilled in the art will realize that biological response modifiers also find use in other human therapies. By way of example, and not limitation, suitable modifiers can be used to treat autoimmune diseases, graft rejection and graft versus host disease in allogeneic bone marrow transplantation, graft rejection in other organ transplants, and infectious disease states such as septic shock.

Depending on the disease to be treated, the immunosuppressive immunotoxin may be used alone or with other immunosuppressive agents. These "cocktails" can be designed to universally and safely suppress immune responses in a wide variety of treatment regimens. Any of a variety of immunosuppressant agents known to the skilled artisan can be combined in the cocktail. A preferred agent is cyclophosphamide at doses ranging from about 50 to 1500 mg/m^2, preferably about 500 mg/m^2/day or about 100 mg/m^2/day for 14 days. The use of cyclophosphamide in conjunction with immunotoxins is disclosed in the commonly assigned application U.S.S.N. 018,324, filed February 24, 1987, and incorporated

herein by reference. Other agents also may be used, for example, prednisone may be utilized at concentrations ranging from about 50 to 250 mg/m^2, preferably about 100 mg/m^2. Similarly, dexamethasone may be used in conjunction with the cyclophosphamide at doses of about 5 to 30 mg, preferably about 15 mg. Typically, cyclophosphamide and the additional immunosuppressive agents will be given coincidentally, such as both at day one, or both on five daily injections, or the like, but alternating administrations may also be utilized. Actual methods for preparing and administering oral and parenteral compositions will be known or apparent to those skilled in the art and are described in detail, e.g., in Remington's Pharmaceutical Science, 16th Ed., Mack Publishing Co., Pennsylvania (1982), which is incorporated herein by reference.

The following examples are offered by way of illustration and not limitation.

EXPERIMENTAL

A. Preparation of RTA-Based Immunotoxins

A preferred production process for RTA-based immunotoxins is described in U.S. Patent No. 4,590,071, which is incorporated herein by reference.

1. Ricin Extraction from Whole Castor Beans.

Whole Castor beans are mechanically ground, and ricin extracted from the meal with a solution of 0.9% saline. This solution was filtered from the bean pellet and lipid layer using a Celite Filter Aid and Aerosil Adsorbent (Manville Denver, CO; Degussa, Frankfurt). The filtrate was concentrated and then diafiltered against Tris Lactose, pH 7.8 (50mM lactose, 10mM Tris pH 7.8, 50mM NaCl), and passed through a QAE ZETA prep cartridge (AMF-Cuno, LKB Instruments, Pleasant Hill, CA). The resultant material was

diafiltered against a Tris saline solution (10mM Tris, 0.9% NaCl, pH 7.8).

2. Ricin Toxin A-Chain Separation.

The diafiltrate was applied to a Sepharose 4B column (Pharmacia Fine Chemicals, Piscataway, NJ) and the nonbinding flow-through containing ricin was loaded onto an acid-treated Sepharose column in order to separate the ricin toxin A-chain from the whole ricin (as described in U.S. 4,590,071, column 3, lines 26-52). The eluant thus obtained was diafiltered against Tris buffer (10mM Tris, 10mM NaCl), and the resulting filtrate was passed through a QAE Sepharose Fast Flow column (Pharmacia Fine Chemicals) equilibrated to the same buffer. The RTA obtained above was adjusted in NaCl concentration to 0.9 wt.%, and purified to remove toxin B-chain impurities by applying to a Sepharose column previously coupled to goat anti-RTB antibodies.

3. Immunotoxin Preparations.

The murine monoclonal antibody XMMLY-H65 is directed against the CD5 antigen, one of the "pan T" antigens, present on 85-100% of human mature T lymphocytes, and on a small population of B lymphocytes. This antigen is not present on hematopoietic progenitor cells nor on any normal adult or fetal human tissue except lymphocytes; and extensive studies by flow cytometry, immunoperoxidase staining, and red cell lysis have not demonstrated binding to such tissues. It is of the IgG1 subclass, and does not fix human or rabbit complement.

The cell line XMMLY-H65 was deposited with the A.T.C.C. and designated Accession No. HB9286. Immunotoxins utilizing that monoclonal antibody were prepared as follows:

An XMMLY-H65 tissue culture harvest was concentrated and the pH adjusted to 8.5. The solution

was applied to an immobilized Staph. Protein A Column and eluted with 0.1 M Citrate, pH 4.5. The eluate was diafiltered against 10mM Hepes Buffer, 0.25 M NaCl, pH 7.3, and then applied to a QAE Sepharose Fast Flow column. The antibody passed through the column, and was diafiltered against PBS, pH 7.0, 5% dextrose. The antibody was activated for coupling to the RTA with SPDP (as described in U.S. 4,590,071, column 4, line 55, column 5, line 5, except that the buffer contained 5% dextrose instead of azide).

A concentrated RTA-30 solution and the antibody solution were placed together in a formulation buffer consisting of 10mM P04, pH 7.0, 0.15 M NaCl, and 5% dextrose. This solution was applied to a Sephacryl S-200 HR column (Pharmacia Fine Chemicals), which had been pre-equilibrated with PBS containing 5% dextrose, and the immunotoxin eluted as fractions (as described in U.S. 4,590,071, column 5, lines 15-24). In some batches, Tween 80 was added up to 0.1% in the final solution.

B. Materials and Methods for Detection of Human Antibodies to Immunotoxin Components

Human IgM and IgG antibody titers to ricin A chain and murine monoclonal antibody XMMLY-H65 were measured by enzyme linked immunosorbant assay (ELISA). Mictrotiter plates (Falcon 3915 Pro-Bind, Becton Dickinson, Oxnard, CA) used to detect antibodies to ricin A chain were prepared by adsorbing ricin A chain (XOMA Corp., Berkeley, CA) diluted in phosphate buffered saline (PBS) [Gibco, Grand Island, NY] at 4°C overnight. After thorough washing in PBS containing 0.05% Tween 20 (PBST) [Sigma, St. Louis, MO] the wells were blocked with 1% glycine (BioRad, Richmond, CA) in PBS at room temperature for 1 hour.

Microtiter plates used to detect antibodies to XMMLY-H65 were prepared by adsorbing XMMLY-H65 (or other antibody) diluted in 0.1M carbonate buffer, pH

9.6, as described above. After thorough washing in PBST, the wells were blocked with 1% bovine serum albumin (BSA) [Sigma, St. Louis, MO] in carbonate buffer at room temperature for 1 hour.

Plates were washed as described above and patient serum samples, appropriately diluted in PBST with 1% BSA (PBST-BSA), were added to triplicate wells. The samples were incubated at 37°C for 1 hour. After washing as described above, either alkaline phosphatase linked goat-human IgM (mu chain specific) [Zymed, South San Francisco, CA] or goat anti-human IgG (gamma chain specific) [Tago, Burlingame, CA], diluted as suggested by the supplier in PBST-BSA, was added and incubated at 37°C for 1 hour. Plates were washed as above followed by washes with distilled water. Substrate (p-nitrophenylphosphate) [Sigma, St. Louis, MO] diluted to 1 mg/ml in 10% diethanolamine buffer was added and plates were incubated at room temperature for 30-60 minutes. Patient immune responses were identified as probe-substrate reactions and were recorded as optical densities at 405nm by a Microelisa Auto Reader (Dynatech, Alexandria, VA).

C. Immune Response Analysis.

The pattern and intensity of the immune response to the murine immunoglobulin and ricin A chain components of the XMMLY-H65-RTA immunotoxin were measured using a standard EIA. Graphs were prepared by plotting OD against serum dilutions. The data used to present the antibody response pattern were derived from a single dilution in the linear portion of the titration curves. The response patterns described in other figures were generated from serum dilutions which most accurately reflect the dynamics of the response. The presence of non-specific or cross-reactive antibody binding activity was noted in some pretreatment sera. At serum dilutions below 1:100 this could obscure interpretation of the immune response data.

To provide a more accurate assessment of the magnitude of the response both the OD values and the dilutions at which they occurred were considered. This was achieved by extrapolating the linear portions of the titration curves to the X axis and reporting the magnitude as a response ratio comparing the maximum end point titer to the pretreatment baseline titer. Twenty normal controls were tested to define the baseline variability in the assay system. In a general for IgG responses, serum dilutions of 1:100 to 1:1000 were required to give baseline OD values less than 0.1. At each dilution, background interference remained relatively constant. In serum samples drawn from 10 normal controls at various intervals, response ratios remained tightly clustered around a value of 1.

D. Patients and Treatment Regimen.

Preliminary evaluation of 45 patients ranging in age from 1 to 47 years entered into a phase I/II study. These patients underwent bone marrow transplantation primarily for hematological malignancies. Twenty-seven patients received histocompatible marrow grafts from sibling donors, and 18 received histoincompatible grafts. (See, Henslee, P. J. et al., J. Exp. Hemat. 15(5) (1987), which is incorporated herein by reference.)

To enter the study, patients had to be refractory to steroid therapy. There were 36 patients with acute graft versus host disease, the majority of which had severe grade III to IV GVHD, and 9 patients had chronic GVHD.

After careful evaluation of toxicity, subsequent patients received escalating doses of immunotoxin ranging from 0.05 to 0.33 mg/kg, daily, for a proposed 14 day course. A number of patients were treated at each dose level, with sixty-seven percent of the patients receiving a full 14 day course and the majority of patients receiving at least 7 or more

doses. All patients were thought to be evaluable for subsequent analysis of toxicity, and those patients who received 7 or greater doses and were evaluable at study day 15 (34 patients) were analyzed for efficacy. This included 25 patients with acute, and 9 patients with chronic, GVHD.

The results obtained from FACS analysis of circulating peripheral lymphocytes prior to therapy and on various study days during and after therapy showed that 12 patients demonstrated a decrease in lymphocytes presenting the mature T cell antigen, CD-3. Overall, there was a rapid decrease in the number of circulating T lymphocytes which reached nadir levels between one and eight days after initiation of immunotoxin treatment. This represents a decreased ability to mount an immune response against antigens presented during this time period. There was no reappearance of T lymphocytes during the remaining treatment days. Between one and three weeks after the end of treatment, the number of T cells slowly returned to baseline.

Preliminary data for 25 patients evaluated in a Phase I/II Clinical Trial with acute graft-versus-host disease, a GVHD grade for each organ was determined on Day 0 prior to therapy, Day 7 and 15 during therapy, and day 28 and 36-45 thereafter. Patients obtaining a complete or partial response in individual organ systems revealed complete or partial resolution of the skin (12/13) or gut (8/11) in patients surviving on day 40, and 7/12 of the patients at this time with liver involvement achieved a complete or partial response.

An Overall Clinical Assessment of the GVHD response was determined utilizing the following categories: complete response, indicated resolution of all signs and symptoms of GVHD; partial response, indicated reduction of at least 1 grade of GVHD severity in any organ system without progression in any other system; mixed response, indicated improvement in

one or more organ system with worsening in another organ; non-response, indicated no improvement; and progression, indicated worsening of GVHD activity in any organ system.

E. Patient Immune Response Suppression

Table II represents a tabulation of data on selected patient's immune response to the two immunotoxin components.

TABLE II

TESTING OF PATIENT SERA IN PHASE 1 ACUTE (A) OR CHRONIC (C) GVHD TRIAL FOR THE PRESENCE OF ANTIBODIES TO XMMLY-H65-RTA

Patient	Patient Dose (mg/kg)	Study Day Of Samples	XMMLY-H65 IgM	XMMLY-H65 IgG	RTA IgM	RTA IgG
VB BMI J01(A)	0.05	1, 9, 15*, 21, 30, 62	+	+	+	+
FC BMI JO2(C)	0.05	1, 7, 15, 62, 97	-	+	-	+
DB BMI NO1(A)	0.05	1, 7, 11, 15	-	-	-	-
MC BMI J03(A)	0.1	1, 6, 10, 14	-	-	-	-
TR BMI 001(A)	0.1	1, 7, 10, 16, 23	-	-	±	-
RJ BMI 002(A)	0.1	1, 6, 11, 14, 26, 29	+	+	+	+
GHD BMI 005(C)	0.1	0, 15, 19, 26	-	-	-	-
JC BMI R07(A)	0.1(Tw)	1, 15, 20	-	-	-	-
WA BMI 006(C)	0.2(Tw)	1, 12, 16, 36, 57	+	+	+	+
RPH BMI 011(A)	0.2	1, 2, 8, 14, 21, 44	-	-	-	-
QS BMI 012(A)	0.2(Tw)	1, 7, 16, 24	-	-	-	-
CL BMI 013(A)	0.2(Tw)	0, 7, 14, 21	-	-	-	-
AS BMI R03(A)	0.2	1, 2, 7, 15, 20, 30, 91	+	+	+	+
BM BMI R04(C)	0.2	1, 4, 7, 15, 20, 27	-	+	-	+
DW BMI R05(A)	0.2(Tw)	1, 2, 7, 24, 28	-	-	-	-
KB BMI R06(A)	0.2(Tw)	1, 7, 15, 20, 23	-	-	-	-
MS BMI J04(A)	0.33	0, 1, 15, 24, 32, 78	+	+	+	+
AB BMI N03(A)	0.33	1, 7, 11, 16, 22	-	-	-	-
KB BMI 008(C)	0.33	1, 8, 12, [illegible]	-	-	-	-
DS BMI R02(A)	0.33	1, 8, 19, 26, 107	-	-	+	-

* Underlining indicates a positive response on that test day.

Tw = 0.1% Tween 80

F. Biological Response Modifier Immunosuppression in Animals.

To assess the in vivo immunosuppressive effectiveness of an immunotoxin of the present invention against a biological response modifier, such as a primary immunotoxin, an animal protection study is performed in rabbits as follows.

A formulation of a primary immunotoxin, XMMME-001-RTA, which is composed of a melanoma specific monoclonal antibody conjugated to ricin toxin A chain (RTA), is prepared as described in U.S. Patent No. 4,590,071. A formulation of XMMLY-H65-RTA immunotoxin is prepared as described above for use as the immunosuppressive immunotoxin.

Full grown adult rabbits are divided into two groups of six rabbits each, and blood drawn from each to determine a baseline antibody titer. All rabbits in each group are then individually innoculated intravenously (iv) with XMMME-001-RTA at a dose of 0.1 mg/kg, daily, for fourteen days. Concurrently, each rabbit in the second group is also individually innoculated iv with XMMLY-H65-RTA at a dose of 0.1 mg/kg, daily for fourteen days. In the control group, no additional innoculations are made. All of the animals are then observed for ninety days, during which blood samples are drawn every six days. The blood samples are analyzed for the pattern and intensity of rabbit IgM and IgG antibodies produced against each of the XMMME-001-RTA immunotoxin components as described above (except that goat-anti-rabbit IgM or IgG are used in the ELISA assay).

The rabbit IgM and IgG response patterns against XMMME-001-RTA are analyzed as above by using a response ratio comparing the maximum end point titer to the pretreatment baseline titer of the antibodies. The control group exibits on average substantially higher response ratios to XMMME-001 and RTA than the second group, which exhibits an average ratio between about 1 and 10 to each of the two components.

From the foregoing it will be appreciated that the immunosuppressive immunotoxins of the present invention provide practical means for patient immunosuppression against biological response modifiers. Importantly, these immunotoxins permit, but do not require, the use of increased and prolonged doses of the modifiers, improving the prognosis of the modifier-based therapy. Moreover, the immunosuppressive immunotoxins do not significantly adversely affect the modifier's effectiveness and do not substantially increase the toxicity of the overall therapy.

Although the present invention has been described in some detail by way of example for purposes of clarity and understanding, it will be apparent that certain changes and modifications may be practiced within the scope of the appended claims.

WE CLAIM:

1. A method for inhibiting an immune response of a patient against an immunogenic biological response modifier comprising administering to the host an immunosuppressive dose of an immunotoxin with said modifier, the immunotoxin comprising an anti-pan T-cell immunoglobulin complexed with a cytotoxic agent.

2. A method according to Claim 1, wherein the biological response modifier is a protein.

3. A method according to Claim 2, wherein the protein is heterologous to the patient.

4. A method according to Claim 1, wherein the cytotoxic agent is a ribosomal inhibiting protein.

5. A method according to Claim 4, wherein the protein is ricin or ricin A-chain.

6. A method according to Claim 1, wherein the immunoglobulin is a mouse monoclonal antibody.

7. A method according to Claim 1, wherein the immunoglobulin is reactive with an epitope on an antigen cluster selected from the group consisting of CD3, CD5, and CD7.

8. A method according to Claim 7, further comprising administering one or more additional immunotoxins comprising immunoglobulins reactive with at least two different T-cell specific markers.

9. A method of Claim 1, wherein the cytotoxic agent is covalently linked to the immunoglobulin.

10. A method according to Claim 9, wherein at least one of the immunotoxins comprises XMMLY H65 monoclonal antibody (A.T.C.C. HB9286) complexed with ricin A-chain.

11. A method for suppressing a patient's immune response to an antigen comprising administering an immunosuppression dose of an immunotoxin composition, which comprises a plurality of immunotoxins, each composed of a delivery component complexed to a toxin component and each reactive with a T-cell marker.

12. A method according to Claim 11, wherein the delivery component is an immunoglobulin.

13. A method according to Claim 11, wherein the marker is located on an antigen cluster.

14. A method according to Claim 11, wherein the antigen cluster is selected from the group consisting of CD2, CD3, CD4, CD5, CD6, CD7, and CD9.

15. A method according to Claim 11, wherein at least one of the delivery components is monoclonal antibodies reactive with a pan T-cell marker.

16. A method according to Claim 11, wherein the immunotoxin comprises XMMLY-H65 monoclonal antibody (A.T.C.C. HB9286) covalently linked to ricin A-chain toxin.

17. A method according to Claim 11, wherein the immunosuppressive dose comprises about 0.01 to 4.0 mg per kg of pateint body weight.

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US89/00439

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int. Cl. A61K 37/00, 37/24,39/00, 39/395,45/02

US Cl. 424/85.91,85.1,85.2,85.8,85.4; 514/2,8,12,21

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
US	424/85.91,85.8,85.1,85.2,85.4,85.5,85.6,85.7,88; 514/2,8,12,21,885

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
X / Y	British Journal of Haematology, Vol. 67, Issued 1987, "Elimination of T-cells from Human Peripheral Blood and Bone Marrow Using a Cocktail of Three Anti-T-Cell Immunotoxin," (Katz), pages 407-11, See page 407.	1,2,4-9,11-15 / 3,10,16-17
X	Blut, Vol. 50, Issued 1985, "Ex Vivo Elimination of of Neoplastic T-Cells from Human Marrow Using an Anti-M_r-41,00 Protein Immunotoxin:Potentiation by ASTA Z7557," (Uckun), pages 19-23, See page 19.	1,4,6,9
X	Cancer Research, Vol. 47, Issued January 1987, "Combined Immunochemotherapy of Human Solid Tumors in Nude Mice," (Weil-Hillman), pages 579-85, See page 579.	1,4-7,9
X	Cancer Research, Vol. 45, Issued November 1985, "Kinetics of Protein Synthesis Inactivation in Human T-Lymphocytes by Selective Monoclonal Antibody-Ricin Conjugates," (Leonard), pages 5263-5269, See page 5263.	1,4-6,9
P,Y	US, A, 4,731,244, (Talle), 15 March 1988, See claims.	1-17

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
29 April 1989	21 JUN 1989
International Searching Authority	Signature of Authorized Officer
	GARNETTE D. DRAPER

Form PCT/ISA/210 (second sheet) (Rev.11-87)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No
Y	US, A, 4,681,760 (Fathman), 21 July 1987, See claims.	1-17
Y	US, A, 4,664,911, (Uhr), 12 May 1987, See claims.	11-17
Y	US, A, 4,590,071, (Scannon), 20 May 1986, See claims.	11-17
X	Journal Immunology, Vol. 136, Issued June 1986, "Antigenic Modulation by Anti-CD5 Immunotoxins" (Manske), pages 4721-28, see page 4721.	1,4-7,9
X / Y	Science, Vol. 222, Issued November 1983, "Anti-T-Cell Reagents For Human Bone Marrow Transplantation:Ricin Linked to Three Monoclonal Antibodies," (Vallera), pages 512-15.	1,2,4-9,11-15 / 3,10,16-17
Y	Pharmac. Ther., Vol. 15, Issued 1982, "Chimeric Toxins," (Olsnes), pages 355-81, See all.	1-17
X / Y	Biological Abstract No. 05915636, "Effects of Therapy With T101 Ricin A Chain Immunotoxin in Two Leukemia Patients," (Laurent), Blood, 67(6), 1986, pages 1680-1687.	1,4-7,9 / 2-3,8,10-17
X / Y	Biological Abstract No. 06176231, Specific Ex-Vivo Depletion of Human Bone Marrow T-Lymphocytes by an Anti-Pan-T-Cell (CD5) Ricin A-Chain Immunotoxin," (Siena), Transplantation, 43(6), 1987, pages 421-6.	1,4-7,9 / 2-3,8,10-17

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Y	Pharmac. Ther., Vol. 15, Issued 1982, "Chimeric Toxins," (Olsnes), pages 355-81, See all.	1-17
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X / Y	Biological Abstract No. 06176231, Specific Ex-Vivo Depletion of Human Bone Marrow T-Lymphocytes by an Anti-Pan-T-Cell (CD5) Ricin A-Chain Immunotoxin," (Siena), Transplantation, 43(6), 1987, pages 421-6.	1,4-7,9 / 2-3,8,10-17